cr oxicative protein modification in relation to ischemia/reperfusion injury and)

L164 ANSWER 28 OF 68 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:439289 CAPLUS
DOCUMENT NUMBER: 127:131510
TITLE: Renal cell regeneration following oxidant exposure: inhibition by TGF-.beta.1 and stimulation by ascorbic acid
AUTHOR(S): Nowak, Grazyna; Schnellmann, Rick G.
CORPORATE SOURCE: Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR, 72205-7199, USA
SOURCE: Toxicol. Appl. Pharmacol. (1997), 145(1), 175-183
CODEN: TXAPA9; ISSN: 0041-008X
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Renal proximal tubular cell (RPTC) monolayers exposed to the model oxidant tert-butylhydroperoxide (TBHP; 0.8 mM) for 1.5 h were 33 and 31% confluent after 1 and 4 days, resp. Control monolayers remained 100% confluent throughout the expt. Exogenous TGF-.beta.1 promoted monolayer deterioration by potentiating cellular death and suppressed EGF-stimulated regeneration of the RPTC monolayer. Net TGF-.beta.1 prodn. in injured RPTC increased 1.7- and 3.2-fold on Days 1 and 2, resp., and returned to control levels 4 days following TBHP treatment. An anti-TGF-.beta. antibody increased monolayer confluence to 50% and DNA content 1.3-fold 4 days after TBHP exposure. L-Ascorbic acid 2-phosphate (AscP) present only during the recovery period increased monolayer confluence to 67% but had no effect on RPTC proliferation, suggesting that AscP promoted monolayer regeneration by cellular migration/spreading. AscP present continuously had no effect on the extent of TBHP-induced injury but promoted regeneration of RPTC with increased monolayer confluence (1.8-fold) and DNA content (1.8-fold) and decreased cellular lysis by 52% 4 days following TBHP exposure. The results demonstrate that TBHP-induced injury increases net TGF-.beta.1 prodn. in RPTC and that autocrine TGF-.beta.1 inhibits regeneration of the monolayer by potentiating cellular injury and monolayer deterioration. The data also show that AscP is not cytoprotective during TBHP exposure but promotes RPTC regeneration by stimulating proliferation and migration/spreading and decreasing cellular death during the recovery period.
IT **50-81-7**, Ascorbic acid, biological studies **23313-12-4**,

Searched by Barb O'Bryen STIC 308-4291

L-**Ascorbic acid** 2-**phosphate**
RL: **BAC (Biological activity or effector, except adverse)**; BIOL (Biological study)
(TGF-.beta.1 and ascorbic acid effect on renal cell regeneration following oxidant exposure)

L164 ANSWER 29 OF 68 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:494349 CAPLUS
DOCUMENT NUMBER: 125:150779
TITLE: Anti-irritant skin formulations containing aluminum or tin cations
INVENTOR(S): Hahn, Gary Scott; Thueson, David Orel
PATENT ASSIGNEE(S): Cosmederm Technologies, USA
SOURCE: PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9619183	A1	19960627	WO 1995-US16765	19951221
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI				

CORPORATE SOURCE: Central Research Laboratories, Santen Pharmaceutical Co., Ltd., Osaka, Japan.
SOURCE: JOURNAL OF OCULAR PHARMACOLOGY, (1994 Fall) 10 (3) 537-42.
Journal code: IRG; 8511297. ISSN: 8756-3320.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950314
Last Updated on STN: 19950314
Entered Medline: 19950227

AB In developing chick embryos, hydrocortisone induces cataract formation following a decrease in lens glutathione content but an increase in lipid peroxide content in lens, blood and liver. The preventive effects of ascorbic acid 2-O-alpha-glucoside (AA-2G) on these parameters were compared on cataract formation with those of ascorbic acid (AsA) and **ascorbic** acid 2-O-**phosphate** (AA-2P). In these tissues, AA-2G inhibited a decrease in glutathione content and an increase in lipid peroxide content more effectively than either AsA or AA-2P. Various tissues including lens and liver have alpha-glucosidase activity, strongly suggesting that AsA is enzymatically liberated from AA-2G in these tissues. In summary, these results suggest that AA-2G exerts a potent anti-cataract activity via a reduction in oxidative damage through AsA release.

L164 ANSWER 2 OF 68 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 93252299 MEDLINE
DOCUMENT NUMBER: 93252299 PubMed ID: 8486304
TITLE: **Ascorbic** acid **phosphate** ester and wound healing in rabbit corneal alkali burns: epithelial basement membrane and stroma.
AUTHOR: Saika S; Uenoyama K; Hiroi K; Tanioka H; Takase K; Hikita M
CORPORATE SOURCE: Department of Ophthalmology, Wakayama Medical College, Japan.
SOURCE: GRAEFES ARCHIVE FOR CLINICAL AND EXPERIMENTAL OPHTHALMOLOGY, (1993 Apr) 231 (4) 221-7.
Journal code: FPR; 8205248. ISSN: 0721-832X.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306
ENTRY DATE: Entered STN: 19930618
Last Updated on STN: 19930618
Entered Medline: 19930607

AB We examined the effect of L-**ascorbic** acid 2-**phosphate** (P-Asc) on the healing of alkali-burned corneas in rabbits. Round filter paper containing 1 N NaOH was applied to the central cornea for 60 or 120 s to produce the alkali burn. Animals were treated with topical saline, 10% ascorbate, or 6.5% P-Asc applied on the cornea. The corneas were then examined histologically. Burned stroma showed no toluidine blue staining, indicating a loss of glycosaminoglycan. In the 60-s burn group, P-Asc reduced the size of the unstained area as compared with the control. Transmission electron microscopy showed basal lamina under new epithelia in the corneas treated with ascorbate or P-Asc, but not in controls. These observations support the theory that P-Asc may have a therapeutic role in the repair of corneal alkali burns.

L164 ANSWER 3 OF 68 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 93217344 MEDLINE
DOCUMENT NUMBER: 93217344 PubMed ID: 8463733
TITLE: Effect of ascorbic acid 2-O-alpha-glucoside on

US005230996A

United States Patent [19]

Rath et al.

[11] Patent Number: 5,230,996

[45] Date of Patent: Jul. 27, 1993

[54] **USE OF ASCORBATE AND TRANEXAMIC ACID SOLUTION FOR ORGAN AND BLOOD VESSEL TREATMENT PRIOR TO TRANSPLANTATION**

[75] Inventors: **Matthias W. Rath**, Kirchberg/Murr, Fed. Rep. of Germany; **Linus C. Pauling**, Big Sur, Calif.

[73] Assignee: **Therapy 2000**, Palo Alto, Calif.

[21] Appl. No.: **556,968**

[22] Filed: **Jul. 24, 1990**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 533,129, Jun. 4, 1990, abandoned.

[51] **Int. Cl.**[5] **A01N 1/02**; A01N 43/08; A01N 33/02

[52] **U.S. Cl.** **435/1**; 514/474; 514/659

[58] **Field of Search** 435/1, 2

[56] **References Cited**

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Harpel, P. C. et al., "Plasmin catalyzes binding of lipoprotein(a) to immobilized fibrinogen and fibrin," Proc. Natl. Acad. Sci. USA 86:3847–3851 (1989).
Gonzalez–Gronow, M. et al., "Further characterization of the cellular plasminogen biding site: Evidence that Plasminogen 2 and Lipoprotein a compete for the same site," Biochemistry 28:2374–2377 (1989).

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Primary Examiner—Douglas W. Robinson
Assistant Examiner—S. Saucier
Attorney, Agent, or Firm—George C. Limbach

[57] **ABSTRACT**

A method and pharmaceutical agent are provided for the prevention and treatment of cardiovascular disease, particularly cardiovascular disease in the context of diabetic angiopathy, by-pass surgery, organ transplantation, and hemodialysis, by administering ascorbate and substances that inhibit the binding of lipoprotein (a) to blood vessel walls. The use of ascorbate and lipoprotein (a) binding inhibitors such as tranexamic acid in a temporary storage solution for blood vessels and organs prior to transplantation is also demonstrated.

8 Claims, 5 Drawing Sheets

USE OF ASCORBATE AND TRANEXAMIC ACID SOLUTION FOR ORGAN AND BLOOD VESSEL TREATMENT PRIOR TO TRANSPLANTATION

This application is a continuation-in-part of application Ser. No. 07/533,129, filed Jun. 4, 1990, now abandoned.

TECHNICAL FIELD

The present invention relates generally to the prevention and treatment of cardiovascular disease arising as a complication from surgery or a pre-existing, unrelated disease, and more particularly to methods and compounds for that inhibit the binding of lipoprotein (a) to components of the arterial wall.

BACKGROUND OF THE INVENTION

Lipoprotein (a) ("Lp(a)") was first identified by Blumberg, B. S., et al. (1962) J. Clin. Invest. 41: 1936–1944 and Berg, K. (1963) Acta Pathol. 59: 369–382. The structure of Lp(a) resembles that of low-density lipoprotein ("LDL") in that both share a lipid-/apoprotein composition, mainly apolipoprotein B-100 ("apo B"), the ligand by which LDL binds to the LDL receptors present on the interior surfaces of arterial walls. The unique feature of Lp(a) is an additional glycoprotein, designated apoprotein(a), apo(a), which is linked to apo B by disulfide groups. The cDNA sequence of apo(a) shows a striking homology to plasminogen, with multiple repeats of kringle 4, one kringle 5, and a protease domain. The isoforms of apo(a) vary in the range of 300 to 800 kDa and differ mainly in their genetically determined number of kringle 4 structures. McLean, J. W., et al. (1987) Nature 300: 132–137. Apo(a) has no plasmin-like protease activity. Eaton, D. L., et al., (1987) Proc. Natl Acad. Sci. USA, 84: 3224–3228. Serine protease activity, however, has been demonstrated. Salonen, E., et al. (1989) EMBO J. 8: 4035–4040. Like plasminogen, Lp(a) has been shown to bind to lysine-sepharose, immobilized fibrin and fibrinogen, and the plasminogen receptor on endothelial cells. Harpel, P. C., et al. (1989) Proc. Natl. Acad. Sci. USA 86:3847–3851; Gonzalez-Gronow, M., et al. (1989) Biochemistry 28: 2374–2377; Miles, L. et al. (1989) Nature 339: 301–302; Hajjar, K. A., et al. (1989) Nature 339: 303–305. Furthermore, Lp(a) has been demonstrated to bind to other components of the arterial wall like fibronectin and glycosaminoglycans. The nature of these bindings, however, is poorly understood.

Essentially all human blood contains lipoprotein (a); however, there can a thousand-fold range in its plasma concentration between individuals. High levels of Lp(a) are associated with a high incidence of cardiovascular disease. Armstrong, V. W., et al. (1986) Atherosclerosis 62: 249–257;Dahlen, G., et al. (1986) Circulation 74: 758–765; Miles, L. A., et al. (1989) Nature 339: 301–302; Zenker, G., et al. (1986) Stroke 17: 942–945 (The term cardiovascular disease will be used hereafter as including all pathological states leading to a narrowing and/or occlusion of blood vessels throughout the body, but particularly atherosclerosis, thrombosis and other related pathological states, especially as occurs in the arteries of the heart muscle and the brain.)

It has also been suggested that Lp(a), the concentration of which increases markedly in the blood during pregnancy, may be linked to cardiovascular disease in woman. Zechner, R., et al. (1986) Metabolism 35: 333–336. It has also been observed that diabetics, many of whom suffer in some degree from atherosclerotic diseases, display greatly elevated serum levels of Lp(a). Bruckert, E., et al. (1990) JAMA 263: 35–36.

Low levels of ascorbate have also been associated with high incidences of cancer (Wright, L. C. et al. (1989) Int. J. Cancer 43: 241–244) and atherosclerosis in diabetes mellitus patients (Som, S. et al. (1981) Metabolism 30: 572–577). In all instance, serum concentrations of Lp(a) were elevated.

In addition to atherosclerosis and thrombosis in arteries Lp(a) has also been linked to stenosis of vein grafts after coronary bypass surgery. Hoff, H., et al. (1988) Circulation 77: 1238–1244. Similar problems of rapid occlusion of vessels have been observed in heart transplant patients.

For some time, general medical practice has focused on the role of LDL, the so called "bad cholesterol," in cardiovascular disease. A great many studies have been published ostensibly linking cardiovascular disease with elevated levels of LDL. As a result, most therapies for the treatment and prevention of arteriosclerosis rely on drugs and methods for the reduction of serum levels of LDLS. Such therapies have had mixed results. The efficacy of such approaches to the problem of cardiovascular disease continues to be major source of debate.

There exists a need, therefore, for a drug and therapy for reducing the binding of Lp(a) to vessel walls, for reducing the overall level of Lp(a) in the circulatory system and for promoting the deposition of existing deposits of Lp(a) on vessel walls.

SUMMARY OF THE INVENTION

The foregoing needs in the treatment and prevention of cardiovascular disease are met by the methods and compositions of the present invention.

A method is provided for the treatment of cardiovascular disease, particularly atherosclerosis, induced or promoted by kidney failure, diabetes, transplant surgery and the like, comprising the step of administering to a subject an effective amount of ascorbate and one or more binding inhibitors, as a mixture or as a compound comprising ascorbate covalently linked with binding inhibitors, which inhibit the binding of Lp(a) to blood vessel walls, such as arterial walls and vein grafts used in by-pass surgery. This effect may also be obtained by administering an effective amount of one or more inhibitors, without ascorbate. The term binding inhibitor throughout the specification and claims is intended to include all substances that have an affinity for the lysine binding site present on the interior walls of blood vessels, particularly arteries, the site of Lp(a) binding. Most of these substances compete with plasmin for the lysine binding site and some of these compounds, in high doses, are in clinical use for the treatment of hyperfibrinolytic states.

A method is further provided for the prevention of atherosclerosis related to or as a complication of surgery, a preexisting disease or a therapy such as hemodialysis. comprising the step of administering to a subject an effective amount of ascorbate and one or more binding inhibitors as previously discussed but further comprising one or more antioxidants. The term antioxidant throughout the specification and the claims is intended to exclude ascorbate, which itself is a powerful antioxidant.

It is thus an object of the invention to provide a method for treatment of induced cardiovascular disease

by administering to a subject an effective amount of ascorbate and one or more binding inhibitors, or an effective amount of one or a mixture of binding inhibitors.

It is another object of the invention to provide a method for prevention of induced cardiovascular disease, by administering to a subject an amount of ascorbate effective to lower the amount of Lp(a) in the plasma of the subject.

Yet another object of the present invention is to provide a method for prevention of induced cardiovascular disease by administering to a subject an effective amount of ascorbate and one or more binding inhibitors, or an effective amount of one or more binding inhibitors.

A further object of the present invention is to provide a pharmaceutically acceptable agent for the treatment of induced cardiovascular disease.

Still another object of the present invention is to provide a pharmaceutically acceptable agent for the prevention of induced cardiovascular disease.

Yet another object of the present invention is to provide a method for preservation of explanted tissues and organs that reduces the risk of occurrence of cardiovascular disease in the tissues and organs after implantation.

It is also an object of the present invention to provide a pharmaceutically acceptable agent to assist in the preservation of explanted tissues and organs prior to implantation.

Still another object of the present invention is to provide a pharmaceutical compound and method for treating cardiovascular disease arising from a preexisting condition of diabetes mellitus.

These and other objects will be more readily understood upon consideration of the following detailed descriptions of embodiments of the invention and the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an immunoblot of the plasma of guinea pigs from the test described in Example 1. Increase of Lp(a) in plasma of guinea pigs with a hypoascorbic diet. Immuunoblot with anti apo(a) antibodies. Lane 1: human control plasma. Lane 2: guinea pig plasma at the start of experiment. Lane 3: guinea pig plasma after 10 days of hypoascorbic diet. Lane 4; guinea pig plasma after 20 days. HMW: high molecular weight standard.

FIG. 2A is a photograph of the aorta of a guinea pig receiving an adequate amount of ascorbate from the test diet in Example 1.

FIG. 2B is a photograph of an aorta of a guinea pig receiving a hypoascorbic diet after three weeks from the test diet in Example 1.

FIG. 3 is an immunoblot of plasma and tissue of guinea pigs from the test shown in Example 2. Plasma and tissue of guinea pigs. Immunoblot with anti apo(a) antibody. HC: human control plasma; L: liver tissue; B: brain tissue; A: aortic tissue, homogenate of plaque area from FIG. 2B.

FIG. 4 is a diagrammatic representation of the action of Lp(a) binding inhibitors on Lp(a) to cause release of Lp(a) from fibrin fibers of an arterial wall.

FIG. 5 is a diagrammatic representation of the action of ascorbate to prevent association and reassociation of Lp(a) to an arterial wall.

DETAILED DESCRIPTION OF THE INVENTION

Our invention is based in part on our discovery that animals which have lost the ability to produce ascorbate, such as higher primates and guinea pigs, uniformly produce Lp(a), whereas most animals which possess the ability to synthesize ascorbate generally do not produce Lp(a). Further, we have found that ascorbate deficiency in humans and guinea pigs tends to raise Lp(a) levels and causes atherosclerosis by the deposition of Lp(a) in the arterial wall, from which we conclude that ascorbate administration lowers plasma Lp(a) levels.

We have also discovered that substances that inhibit the binding of Lp(a) to components of the arterial wall, particularly to fibrinogen, fibrin and fibrin degradation products herein identified as binding inhibitors, such as lysine or ϵ-aminocaproic acid. Thus, ascorbate and such binding inhibitors are not only useful for the prevention of cardiovascular disease, but also for the treatment of such disease.

Some beneficial effects of ascorbate in the prevention and treatment of cardiovascular disease have been established (see FIG. 2). Our invention reveals the relation to and therapeutic use of ascorbate for Lp(a), one of the most atherogenic lipoproteins, directly related to the development of atherosclerotic plaques. The beneficial effects of ascorbate suggest that ascorbate therapies would be useful in a variety of situations where occlusion of blood vessels by Lp(a) deposition is a problem. For instance, ascorbate may be useful in transplantation of blood vessels and whole organs, where a combination of tissue damage to the transplant, such as by oxidation, and high serum Lp(a) in the transplant recipient results in rapid occlusion of blood vessels in the transplant. Ascorbate may also be useful in the area of hemodialysis, where loss of ascorbate and other vitamins and trace elements from the blood of hemodialysis patients can result in increased serum levels of Lp(a) and thus increased risk of cardiovascular disease. Finally, it appears that ascorbate alone and in combination with binding inhibitors, specifically with plasmin competitors, may be therapeutically useful for treatment of the pathogenic effects of diabetes which is associated with elevated serum concentrations of Lp(a).

The present invention provides methods and pharmaceutical agents for both the treatment and prevention of cardiovascular disease in vivo; methods and agents for the preservation of damage linked vessel occlusion in explanted tissues and organs, as well as methods and agents for the prevention of hemodialysis-linked cardiovascular disease. Each of these embodiments is discussed in turn below.

GENERAL APPLICATIONS

The present invention provides a method and pharmaceutical agent for the treatment and prevention of cardiovascular disease generally, particularly atherosclerosis, by administering to a subject an effective amount of ascorbate and one or more binding inhibitors which inhibit the binding of Lp(a) to blood vessel wall components, particularly to fibrin or fibrinogen. As used herein, the term "ascorbate" includes any pharmaceutically acceptable salt of ascorbate, including sodium ascorbate, as well as ascorbic acid itself. Binding inhibitors include, but are not limited to ϵ-aminocaproic acid (EACA), lysine, tranexamic acid (4-aminomethylcyclohexane carboxylic acid), p-aminomethylbenzoic

acid, p-benzylamine sulfuric acid, and α-N-acetyl-lysine-methyl ester and PROBUCOL (a compound comprised of 2 butyl hydroxy tocopherol groups linked together by a disulphide group), Aprotinin, trans-4-aminomethylcyclohexanecarboxylic acid (AMCA), and benzamidine derivatives such as amidinophenylpyruvic acid (APPA) and 1-naphthyl-(1)-3-(6-amidinonaphthyl-(2))-propanone-1 HCl (NANP). An effective amount of a binding inhibitor or a mixture of binding inhibitors may also be used, without ascorbate. Other substances used in the treatment of cardiovascular disease may be co-administered, including: antioxidants, such as tocopherol, carotene and related substances; vitamins; provitamins; trace elements; lipid-lowering drugs, such as hydroxy-methyl-glutaryl coenzyme A reductase inhibitors, nicotinic acid, fibrates, bile acid sequestrants; and mixtures of any two or more of these substances.

Although ascorbate can be used alone or in varying combinations with one or more representative constituents of the above classes of compounds, we prefer when treating a pre-existing cardiovascular condition to combine ascorbate with at least one each of the binding inhibitors, antioxidants and lipid lowering drugs elements in the dosages (per kilogram of body weight per day (kg BW/d)) provided in Table 1. It should be noted that Table 1 provides differing concentration ranges of each constituent, depending upon whether the agent is to be administered orally or parenterally. The variance in dosages is reflective of variation in disease severity. It will be realized therefore that if the subject has been diagnosed for advanced stages of atherosclerosis, dosages at the higher end of this range can be utilized. However, if only prevention of an atherosclerosis condition is the object, dosages at the lower end of this range can be utilized.

As an alternative, a pharmaceutical agent identical to the one just described, but omitting ascorbate, may be employed.

Where ascorbate and binding inhibitors are utilized in the same agent, they may simply be mixed or may be chemically combined using synthesis methods well known in the art, such as compounds in which ascorbate and the inhibitor are covalently linked, or form ionically bound salts. For example, ascorbate may be bound covalently to lysine, other amino acids, or ϵ-aminocaproic acid by ester linkages. Ascorbyl ϵ-aminocaproate is such an example. In this form the ascorbate moiety may be particularly effective in preventing undesirable lipid peroxidation.

In the case of oral administration, a pharmaceutically acceptable and otherwise inert carrier may be employed. Thus, when administered orally, the active ingredients may be administered in tablet form. The tablet may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid, and/or a lubricant such as magnesium stearate. If administration in liquid form is desired, sweetening and/or flavoring agents may be used. If administration is by parenteral injection, in isotonic saline, a phosphate buffered solution or the like, may be used as pharmaceutically acceptable carrier.

The advisability of using binding inhibitors in treating cardiovascular disease will depend to some extent on the subject's general health, particularly with regard to hyperfibrinolytic conditions. Most binding inhibitors (except lysine) are used clinically to treat such conditions. As a result, monitoring of the subject's coagulation and fibrinolytic system is recommended before and during treatment for cardiovascular disease. Long-term administration of binding inhibitors will require formulations in which the dosages of binding inhibitors are in the lower ranges of the dosages given in Table 1.

Prevention, as contrasted with treatment, of cardiovascular disease may be accomplished by oral or parenteral administration of ascorbate alone. Table 1 gives a range of ascorbate concentrations sufficient to lower the serum Lp(a) concentration. Preferably the prevention of the cardiovascular disease according to the invention is accomplished by use of a physical mixture of ascorbate and one or more binding inhibitors, or by use of a compound comprising covalently linked ascorbate with one or more of the binding inhibitors, which inhibit binding of Lp(a) to the arterial wall. A binding inhibitor or mixture of binding inhibitors may also be administered without ascorbate to prevent Lp(a)-associated cardiovascular disease.

To optimize the therapeutic effect of the release of Lp(a) from the blood vessel walls, the ascorbate and the binding inhibitors described above may be separately administered. Further optimization of therapeutic effect can be gained by using a time release composition to achieve relatively constant serum concentrations of the agent through time.

CORONARY BYPASS APPLICATIONS

As discussed above, recurrence of cardiovascular disease after bypass surgery is a frequent problem. Physicians often observe that the veins used to replace occluded arteries become rapidly occluded themselves after implantation, often requiring the patient to undergo successive surgical episodes to replace clogged bypasses. While not wishing to be bound to any theory, we believe that the rapid occlusion observed in many individual's results from a combination of the patient's pre-existing elevated levels of Lp(a) and injury to the bypass veins, during transplantation, particularly as a result of oxidative damage during explantation. This damage makes binding of Lp(a) to the vessel interior easier. Further, Lp(a) has been detected in abundance in reoccluded by-pass veins after coronary bypass surgery. See, Cushing, et al. (1989) Atherosclerosis 9:593–603. Lp(a) is now known to be the most significant factor for reocclusion of bypass veins. See, Hoff, H, et al. (1988) Circulation 77:1238–1244. Thus, a further embodiment of this invention includes using the pharmaceutical agent of the present invention to lower the bypass patient's Lp(a) before, during and after surgery while at the same using a solution containing the agent to rinse and store the bypass veins until such time as the veins are implanted into the recipient, thereby reducing oxidative damage that can make Lp(a) binding more likely after implantation.

The treatment protocols for the bypass patient generally follow those described above for the treatment of pre-existing cardiovascular disease. The composition of the pharmaceutical agent will generally include ascorbate, one or more binding inhibitors, one or more antioxidants and one or more lipid lowering drugs as enumerated and in the dosages given in Table 1. Of course, the level of dosage will depend on disease severity. Further, the constituents of the agent can be combined just as described above, can be administered either orally or parenterally and can be combined with a pharmaceutically acceptable carrier.

TABLE 1

DOSAGES OF COMPONENTS IN THE DRUG COMPOSITIONS OF THE PRESENT INVENTION

	Oral Administration	Parenteral Administration
Ascorbate:	5 mg–2500 mg/kg bw/d	25 mg–2500 mg/kg bw/d
Binding inhibitors:		
EACA	5 mg–500 mg/kg bw/d	same
Tranexamic Acid	1 mg–100 mg/kg bw/d	same
Para-aminomethyl benzoic acid	1 mg–30 mg/kg bw/d	same
Lysine	5–500 mg/kg bw/d	same
Antioxidants:		
Tocopherol	0,1 IU–20 IU/kg bw/d	same
Carotene	100 IU–1000 IU/kg bw/d	same
Lipid Lowering Drugs:		
Nicotinic Acid	1 mg–300 mg/kg bw/d	
HMG-CoA	0.1–10 mg/kg bw/d	
Fibrates	0.1–20 mg/kg bw/d	
Probucol	0.1–20 mg/kg bw/d	
Bile Acid Sequestrants	10–400 mg/kg bw/d	

Turning now to vessel treatment and storage, it is important to provide an in vitro environment which minimizes vessel injury. We conclude that vessel injury can be reduced by the addition of a combination of ascorbate, binding inhibitors and antioxidants to the solution in which the vessels are normally stored. A range of effective concentrations of these constituents in solution is given in Table 2. The general aspects of live vessel preservation and storage prior to implantation are well known in the art.

TABLE 2

CONCENTRATION OF COMPONENTS IN THE SOLUTION OF THE PRESENT INVENTION

Ascorbate	50-5000 mg/l
Binding Inhibitors:	
EACA	2–2000 mg/l
Tranexamic Acid	1–300 mg/l
Para-aminomethyl benzoic acid	1–200 mg/l
Lysine	10–5000 mg/l
Antioxidants	
Tocopherol	1–1000 mg/l
Carotene	0.1–100 mg/l

APPLICATIONS IN ORGAN TRANSPLANTS

We have also found that the solution and method of the present invention are effective in preventing cardiovascular disease from occurring in transplanted organs that have been otherwise successfully implanted in an organ recipient, particularly in the case of the heart.

As with occlusion of transplanted veins after bypass surgery, a transplanted heart free of any substantial arterial occlusion may suffer accelerated atherosclerosis after implantation. We believe that the mechanism described for occlusion of transplanted vessels applies equally to the heart itself as a whole, namely that the heart muscle itself, as well as the interiors of the arterial walls become damaged, making the arteries of the heart more prone to binding with Lp(a). Because the organ recipient often presents elevated serum concentration of Lp(a), particularly after surgery (see, Maeda, S. et al. (1989) Atherosclerosis 78: 145–150), atherosclerosis can proceed at an accelerated rate.

Treatment follows along the same line as that described for bypass surgery. Damage to the organ itself is minimized by placing the organ in a solution containing a mixture of ascorbate, binding inhibitors, and antioxidants in an otherwise standard storage solution. Concentration ranges for the various components in the final solution are given in Table 2. Because of the oxidative cellular damage during extended periods of explantation, the concentration of antioxidants should be in the higher range of dosages disclosed in Table 1. The standard storage solution itself is well known in the art. Storage of the organ in this solution will tend to minimize damage to arterial walls, thereby providing fewer places for Lp(a) to bind.

Of course, patient treatment is also desireable. If the organ recipient suffers from some degree of atherosclerosis at the time of organ transplant, the protocol and drug described above generally for the treatment of atherosclerosis should be employed. If, however, the patient does not suffer from atherosclerosis, use of the drug and protocol described above for prevention of atherosclerosis is desired. In all cases, the lowest dosages of ascorbate should be employed in the drug composition since ascorbate has an immune stimulatory effect.

APPLICATIONS IN HEMODIALYSIS TREATMENT

It is well known that patients who suffered renal failure and require regular dialysis treatment to cleanse the blood of metabolic waste products are also at an increased risk for cardiovascular disease. We believe that the reason for this may be a depletion of ascorbate, vitamins in general and other essential substances from the blood supply during the hemodialytic process. As described more fully above, the loss of ascorbate would result in greater injury to the interior of the artery walls over time and may also result in the production of elevated Lp(a) levels in the blood serum.

As can appreciated, the solution and method of the present invention can be applied both to the patient and the hemodialysis solution to prevent and control hemodialysis-related cardiovascular disease. Turning first to the hemodialysis solution, it is desired to add a combination of ascorbate, binding inhibitors and antioxidants to the solution to produce concentrations of these compounds in solution in the range of concentrations provided in Table 2.

In order to achieve the best results, treatment of the dialysis patient should be carried out in addition to modification of the hemodialysis solution. Treatment

should follow the drug and protocols set forth in detail above for the treatment of a preexisting atherosclerotic condition.

APPLICATIONS IN TREATMENT OF DIABETES

The composition and method of the present invention are also useful in the treatment of the pathological effects of diabetes mellitus. In diabetes mellitus, pathological charges in the arteries frequently lead to clinical symptoms or complete failure in various organs such as the kidney, eye and peripheral circulation system. Therefore, one therapeutic focus in diabetes mellitus is the treatment of diabetic angiopathy.

It appears that glucose competitively inhibits the physiologic uptake of ascorbate in different cell systems of the body, including the arterial wall. Kapeghian, et al. (1984) Life Sci. 34: 577. Such damage to arterial walls creates binding sites for Lp(a). Further, Lp(a) has been found to be elevated in the blood serum of diabetic patients. The atherogenic process is perhaps therefore accelerated by the combination of damaged arteries and elevated Lp(a). Therefore, we propose that ascorbate alone or in combination with at least one binding inhibitor has therapeutic value in treating diabetes-related atherosclerosis.

Thus, another embodiment of the present invention is the use of a composition and method in treating the pathogenic effects of diabetes mellitus, particularly with regard to atherosclerotic conditions.

The treatment protocol involves the oral or parenteral administration of a pharmaceutical composition comprised of ascorbate, one or more binding inhibitors and one or more antioxidants. Dosages for a course of treatment are provided in Table 1. The dosage of ascorbate should preferably fall within the higher range, thereby increasing its chance of cellular uptake in the presence of high serum levels of glucose.

EXPERIMENTAL

Having disclosed the preferred embodiment of the present invention, the following examples are provided by way of illustration only and are not intended to limit the invention in any way.

EXAMPLE 1

Because of its metabolic similarity to man, with respect to the metabolism of ascorbate and Lp(a), the guinea pig was used in this example.

No study has been previously reported in the guinea pig to identify the lipoprotein involved as risk factors in plasma and as constituents of the atherosclerotic plaque.

Three female Hartly guinea pigs with an average weight of 800 g and an approximate age of 1 year were studied. One animal received an extreme hypoascorbic diet with 1 mg ascorbate/kg body weigh'/d. Another animal received 4 mg/k BW/d. The third animal served as a control receiving 40 mg ascorbate/Kg BW/d.

Blood was drawn by heart puncture from the anesthetized animals and collected into EDTA containing tubes at the beginning, after 10 days, and after 3 weeks, when the animals were sacrificed. Plasma was stored at −80° C. until analyzed. Lp(a) was detected in the plasma of the guinea pigs by use of SDS-polyacrylamide gels according to Neville (*J. Biol. Chem.*, 246, 6328–6334 (1971)) followed by Western blotting (Beisiegel, et al., *J.Biol. Chem.*, 257, 13150–13156 (1982)). 40 μl of plasma and 20 mg of arterial wall homogenate were applied in delipidated form per lane of the gel. The immunodetection of apo(a) was performed using a polyclonal anti-human apo(a) antibody (Immuno, Vienna, Austria) followed by a rabbit anti-sheep antibody (Sigma) and the gold-conjugated goat anti-rabbit antibody with subsequent silver enhancement (Bio-Rad). The determinations of cholesterol and triglycerides were done at California Veterinary Diagnostics (Sacramento) using the enzyme assay of Boehringer Mannheim. Plasma ascorbate was determined by the dinitrophenylhydrazine method (Schaffer, et al., *J. Biol. Chem.*, 212, 59 (1955)).

Vitamin C deficiency in the diet led to an increase of Lp(a) in the plasma of the guinea pig indicated by a clear band in the immunoblot of the plasma after 10 and 20 days of a hypoascorbic diet (FIG. 1). At necropsy the animals were anesthetized with metophase and were exsanguinated. Aorta, heart and various other organs were taken for biochemical and histological analysis. The aorta was excised, the adventitial fat was carefully removed, and the vessel was opened longitudinally. Subsequently the aorta was placed on a dark metric paper and a color slide was taken. The picture was projected and thereby magnified by an approximate factor 10. The circumference of the ascending aorta, the aortic arch and thoracic aorta as well as the atherosclerotic lesions in this area were marked and measured with a digitalized planimetry system. The degree of atherosclerosis was expressed by the ratio of plaque area to the total aortic area defined. The difference in the 3 one-year old animals of the experiment was significant and pronounced lesions were observed in the ascending aorta and the arch of the vitamin C deficient animal (FIG. 2B).

EXAMPLE 2

To confirm the data obtained in Example 1, a second guinea pig experiment was conducted, using 33 male animals with a mean weight of 550 g and an approximate age of 5 months. One group of 8 animals served as a control and received 40 mg ascorbate/kg BW/d (group A). To induce hypoascorbemia 16 animals were fed 2 mg ascorbate/kd/d (group B). Group A and half of the animals of group B (progression sub-group) were sacrificed after 5 weeks as described above. Half of group B was kept for 2 more weeks, receiving daily intraperitoneal injection of 1.3 Na-ascorbate/kg BW/d as a daily intra peritoneal injection with the intention to reduce the extent of atherosclerosis lesions. After this period these animals also were sacrificed.

Plasma ascorbate levels were negatively correlated with the degree of the atherosclerotic lesion. Total cholesterol levels increased significantly during ascorbic acid deficiency (Table 3).

The aortas of the guinea pigs receiving a sufficient amount of ascorbate were essentially plaque free, with minimal thickening of the intima in the ascending region. In contrast, the ascorbate-deficient animals exhibited fatty streak-like lesions, covering most parts of the ascending aorta and the aortic arch. In most cases the branching regions of the intercostal arteries of the aorta exhibited similar lipid deposits. The difference in the percentage of lesion area between the control animals and the hypoascorbic diet animals was 25% deposition of lipids and lipoproteins in the arterial wall.

TABLE 3

MEAN PLASMA PARAMETERS OF THE DIFFERENT GROUPS IN RELATION TO THE AREA OF AORTIC LESIONS

	Control	Scurvy (progress)	Regression (after Scurvy)
Plasma Cholesterol (mg/dl)	39	54	33
Total Plasma Ascorbic Acid µg/ml	5.03	3.01	20.64
Atheroscl. Lesion (Percent of Aorta Thorac. Surface)	—	25	19

A possible inhibitor may be identified first by adding molar amounts of the possible inhibitor at a little larger, by approximately 5 times, the amount of ϵ-aminocaproic acid found in the earlier study. If, at this concentration, a possible inhibitor is found to inhibit the agglutination, studies are made at lower concentrations, to determine the concentration that has a 50% initiatory effect.

EXAMPLE 3

Human arterial wall was obtained post mortem from the aorta ascendens. The tissue showed homogenous intimal thickening (early atherosclerotic lesion). It was cut into pieces, with 100 mg of the cut up tissue homogenized in a glass potter for 1 minute in 2.5 ml of the following solutions:

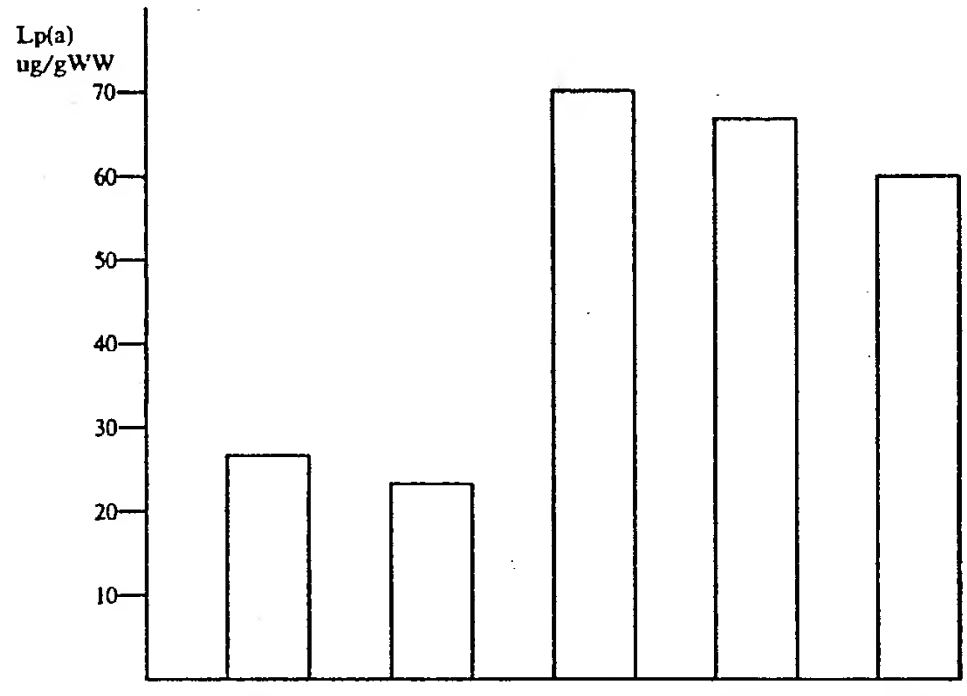
PBS (Dulbeco) + NaAscorbate	50 mg/ml
PBS + EACS	50 mg/ml
PBS + Tranexamic Acid	50 mg/ml
PBS + NaAscorbate + Tranexamic Acid	50 mg/ml

-continued

Results of this treatment are given in Table 4 and show that, compared to the control solution, a considerable amount of Lp(a) was released from the interior arterial wall.

TABLE 4

Lp(a) RELEASED FROM HUMAN AORTA IN RELATION TO SPECIFIC BINDING INHIBITORS



By now it is apparent that the methods and compositions of the present invention meet longstanding needs in the field of prevention and treatment of induced cardiovascular disease. Although preferred embodiments and examples have been disclosed, it is understood that the invention is in no way limited thereby, but rather is defined by the claims that follow and the equivalents thereof.

What is claimed is:

1. A method for reducing lipoprotein(a) binding to vessel explants prior to implantation comprising the step of storing the vessel explants in an aqueous composition comprising ascorbate and tranexamic acid in concentrations sufficient to decrease binding of lipoprotein(a) to interior walls of the vessel explants.

2. A method according to claim 1 wherein said ascorbate is selected from the group consisting of pharmaceutically acceptable ascorbate salts, ascorbic acid and mixtures thereof.

3. A method according to claim 2 wherein said ascorbate is covalently linked to said tranexamic acid.

4. A method for reducing injury to organ explants prior to implantation comprising the step of storing the organ explants in an aqueous composition comprising ascorbate and tranexamic acid in concentrations sufficient to decrease binding of lipoprotein(a) to interior walls of vessels within the organ explants.

5. A method according to claim 4 wherein said ascorbate is selected from the group consisting of pharmaceutically acceptable ascorbate salts, ascorbic acid and mixtures thereof.

6. A method according to claim 5 wherein said ascorbate is covalently linked to said tranexamic acid.

7. A method according to either claim 4 or 5 wherein said composition is a pharmaceutical composition administered in an amount effective to release at least some of the vessel-bound lipoprotein(a).

8. A method for reducing lipoprotein(a) binding to organ explants prior to implantation comprising the step of storing the organ explants in an aqueous composition comprising ascorbate and tranexamic acid in concentrations sufficient to decrease lipoprotein(a) binding to interior walls of vessels within the organ explants.

* * * * *

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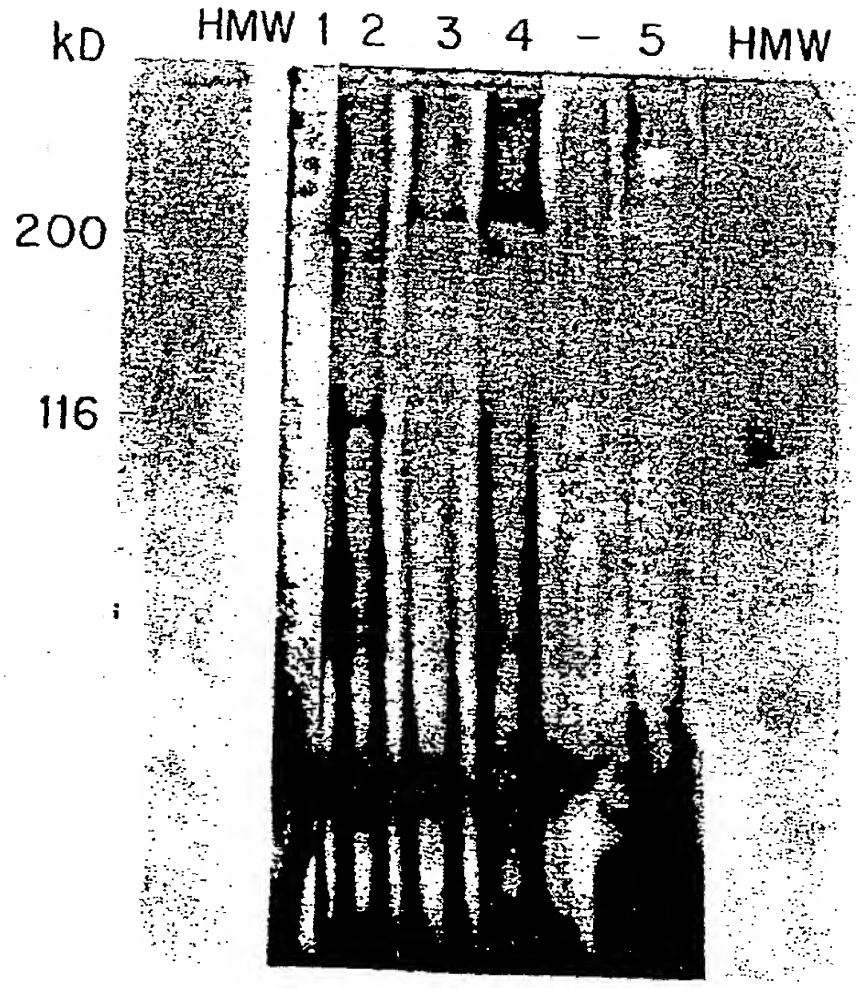

FIG. 1

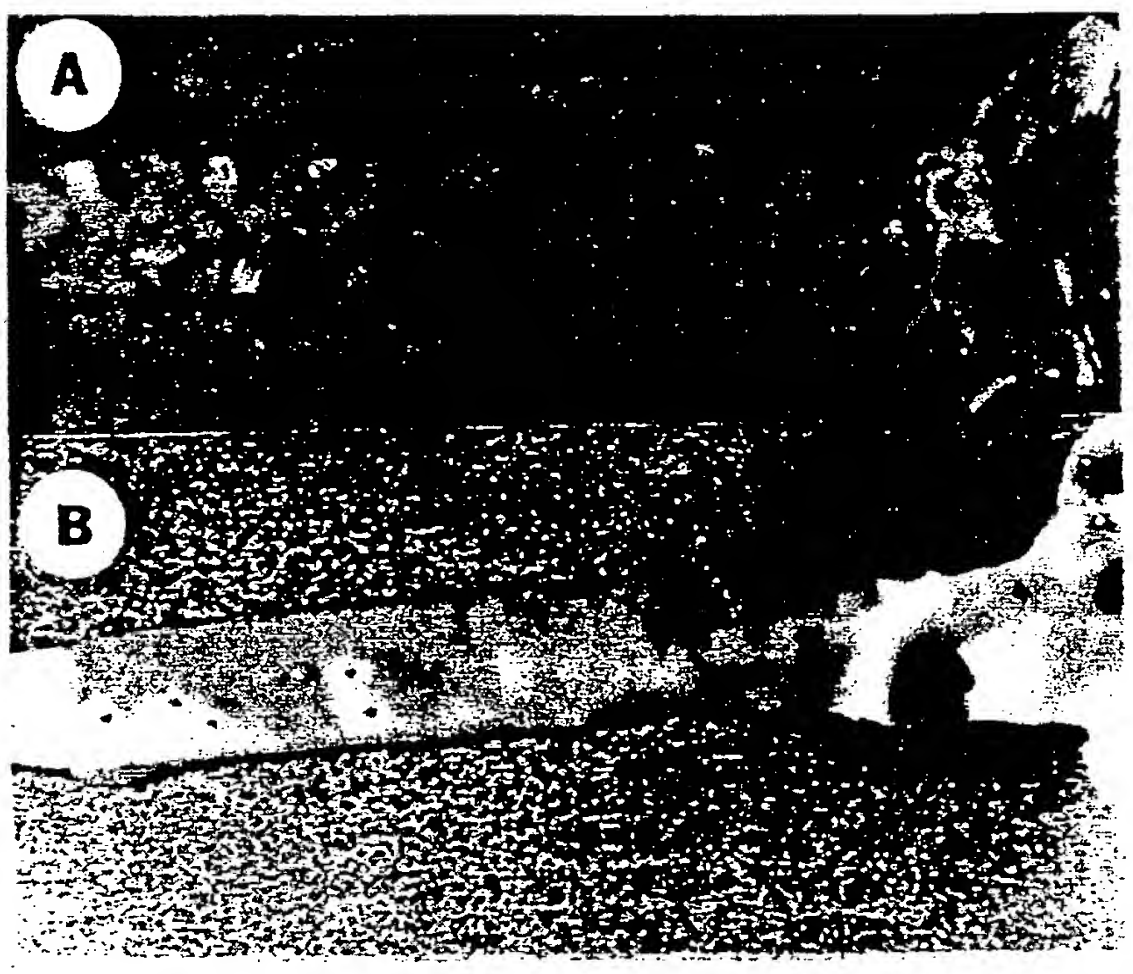

FIG. 2

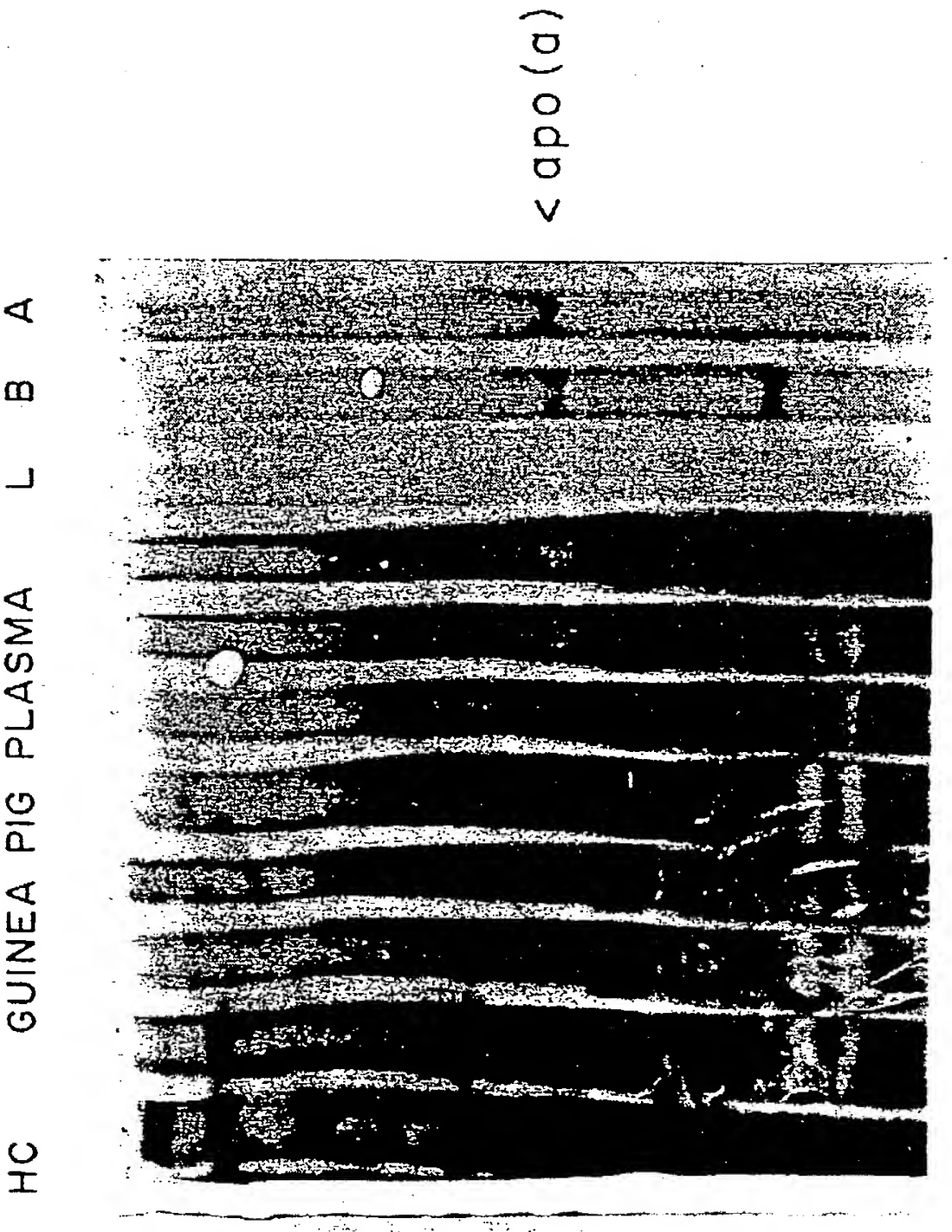

FIG. 3

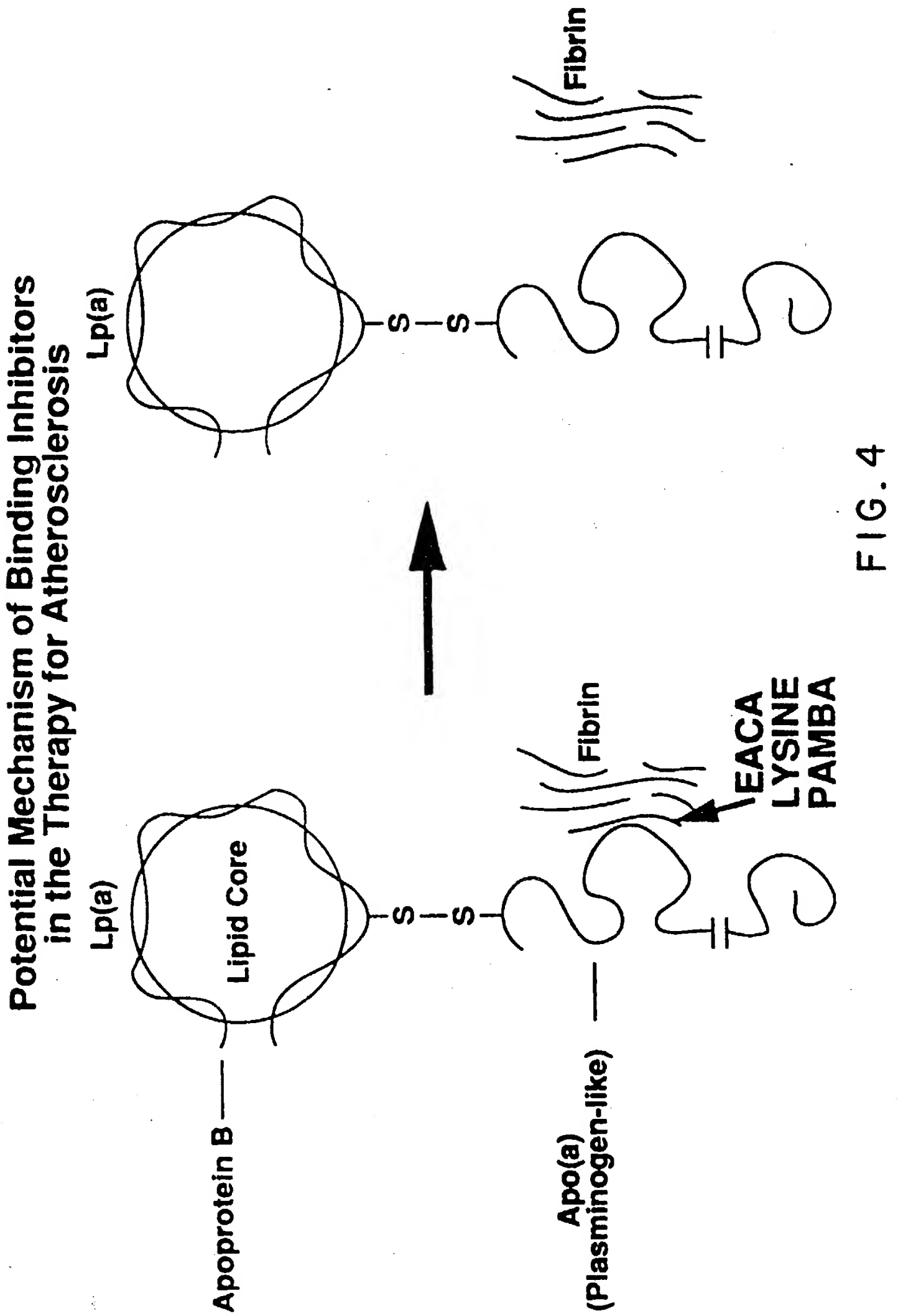
Potential Mechanism of Binding Inhibitors in the Therapy for Atherosclerosis
Lp(a)
Apoprotein B
Lipid Core
S—S
Apo(a)
(Plasminogen-like)
Fibrin
EACA
LYSINE
PAMBA
Lp(a)
S—S
Fibrin

FIG. 4

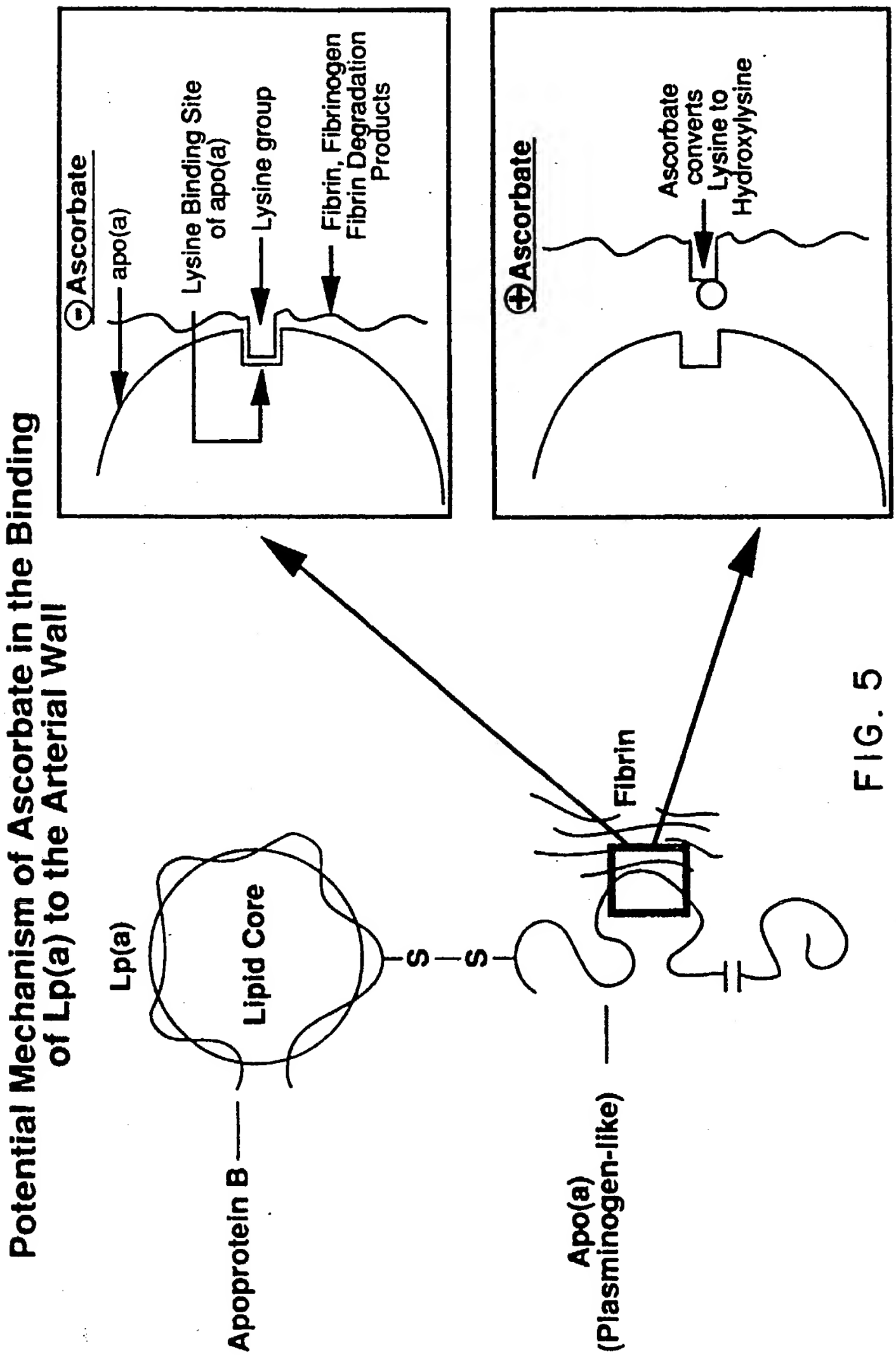
Potential Mechanism of Ascorbate in the Binding of Lp(a) to the Arterial Wall
⊖Ascorbate
apo(a)
Lysine Binding Site of apo(a)
Lysine group
Fibrin, Fibrinogen Fibrin Degradation Products
⊕Ascorbate
Ascorbate converts Lysine to Hydroxylysine
Lp(a)
Lipid Core
Apoprotein B
S—S
Apo(a) (Plasminogen-like)
Fibrin

FIG. 5

United States Patent ***4,711,780***
Fahim **December 8, 1987**

Composition and process for promoting epithelial regeneration

Abstract

A medication for treating the surface epithelium is disclosed comprising vitamin C, a zinc salt and a sulfur amino acid. In some cases, the medication may additionally contain a mucopolysaccharide and/or a polysaccharide. A method of stimulating cell proliferation and new cell formation with said medication is also disclosed.

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Intern'l Class: A61K 033/30; A61K 031/195
Field of Search: 424/145 514/562

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Primary Examiner: Schenkman; Leonard
Attorney, Agent or Firm: Fishel; Grace J.

Parent Case Text

This is a continuation-in-part of application Ser. No. 619,004, filed June 11, 1984 for Treatment of Epithelial and Connective Tissue and Composition Therefor, which was a continuation-in-part of application Ser. No. 341,544, filed Jan. 21, 1982 for Treatment of Vaginitis and Cervicitis, both now abandoned.

Claims

What is claimed is:

1. A medication for treating epithelial tissue comprising vitamin C, a zinc salt and a sulfur amino acid in an amount sufficient to stimulate cell proliferation and new cell formation.
2. The medication of claim 1 further including a mucopolysaccharide.
3. The medication of claim 1 further including a polysaccharide.
4. The medication of claim 1 wherein the sulfur amino acid is selected from the group consisting of cysteine, cystine and methionine.
5. The medication of claim 4 wherein the vitamin C is present in an amount from 0.5 to 30% by weight, the zinc salt is present in an amount from 0.5 to 20% by weight as zinc sulfate heptahydrate or the equivalent amount of zinc present as some other zinc salt, and 0.25 to 5% by weight of sulfur amino acid.
6. The medication of claim 5 further including a mucopolysaccharide selected from the group consisting of chrondroitin sulfate and hyaluronic acid.
7. The medication of claim 5 further including a mucopolysaccharide selected from the group consisting of heparin calcium salt or dermatan sulfate.
8. The medication of claim 5 further including keratan sulfate.
9. A method of treating epithelial tissue with a medication including vitamin C, a zinc salt and a sulfur amino acid in an amount sufficient to stimulate cell proliferation and and new cell formation which comprises applying said medication to the treatment area.
10. The method of claim 9 wherein the medication further includes a mucopolysaccharide.

11. The method of claim 9 wherein the medication further includes a polysaccharide.
12. The method of claim 9 wherein the sulfur amino acid is selected from the group consisting of cysteine, cystine and methionine.
13. The method of claim 9 wherein the medication comprises 0.5 to 30% by weight vitamin C, 0.5 to 20% by weight zinc present as zinc sulfate heptahydrate or the equivalent amount of zinc present as some other zinc salt and 0.25 to 5% by weight of sulfur amino acid.
14. The method of claim 13 wherein the medication further includes a mucopolysaccharide selected from the group consisting of chrondroitin sulfate and hyaluronic acid.
15. The method of claim 13 wherein the medication further includes a mucopolysaccharide selected from the group consisting of heparin calcium salt and dermatan sulfate.
16. The method of claim 13 wherein the medication further includes keratan sulfate.

Description

The present invention relates to a composition for treating the surface epithelium and to a process for promoting epithelial regeneration.

Epithelial tissue covers the entire body, including the internal surfaces of the gastrointestinal tract, genitourinary tract, respiratory tract and reproductive tract and serves to protect the body against injury. When the epithelium is injured, it is well known that the process of healing is complex and begins with cell migration, division, differentiation and production of special products. In some cases, granulation tissue is formed to fill the gap between the edges of the wound with a thin layer of fibrinous exudate consisting of epithelial cells, fibroblasts, endothelial cells, blood-borne cells (e.g., macrophages, lymphocytes, neutrophils and platelets), collagen and glycosanimoglycans. The process of wound healing includes homeostasis (i.e., processes through which bodily equilibrium is maintained), angiogenesis (i.e. production of blood vessels) and fibroplasia (i.e. production of fibrous tissue). The key to understanding the healing process resides in the interaction among the forces of healing, coagulation and inflammation. When the

result of healing closely approximates the normal state the process is referred to as regeneration.

When the epithelium is injured, the amount of zinc, vitamin C, cystine and many other nutrients is reduced in the affected cells. Up until the present time, however, it was not known that the topical application of a combination of zinc salt, vitamin C and sulfur amino acid would be particularly effective at promoting epithelial regeneration.

In view of the above, it is an object of the present invention to provide a composition which promotes epithelial regeneration with a unique combination of materials which, in its preferred form, are present in epithelial tissue at very low levels. Other objects and features will be in part apparent and in part pointed out hereinafter. The invention accordingly comprises the compositions and methods hereinafter described, the scope of the invention being indicated by the subjoined claims.

In accordance with the present invention, a mixture of zinc salt, vitamin C and sulfur amino acid is formed for addition to a pharmaceutical carrier. For use herein, the zinc is provided in a salt form wherein the anion is non-toxic to the subject such as sulfate. The vitamin C may be provided as ascorbic acid, sodium ascorbate or the like and cystine may be replaced with other sulfur amino acids which inhibit collaginase and in general is selected from the group consisting of cystine, cysteine, methionine and di- and tripeptides such as glutathione formed therefrom. A mucopolysaccharide may be included for some applications. Suitable mucopolysaccharides are extracted from animal or plant connective tissue with different mucopolysaccharides being preferred depending on the nature of the epithelium being treated. For example chrondroitin sulfate and hyaluronic acid are preferred in the reproductive tract, heparin calcium salt, dermatan sulfate or mucopolysaccharides extracted from aloe vera plant are preferred for skin ulcerations and keratan sulfate is preferred for treatment of the eyes.

A polysaccharide may also be included in the formulation if the surface to be treated is slippery. Suitable polysaccharides include agar, algin, carboxymethylcellulose, carrageenan, guar gum, gum arabic, gum ghatti, gum tragacanth, hydroxyethylcellulose, hydroxypropylguar, karaya gum, locust bean gum, methylcellulose, pectin and xanthan gum.

In accordance with the present invention, the zinc is present in an amount from about 0.25 to 20% by weight as zinc sulfate heptahydrate or the equivalent amount of zinc present as some other zinc salt. As shown in the examples, the preferred amount of zinc depends on the condition being treated. For example, when the medication is a douche for treating vaginitis or cervicitis and the subject is a menstruating female, the zinc is preferably present in an amount from about 1 to 2% by weight but when the subject is pre- or postmenopausal, less zinc is required with the preferred amount being from about 0.25 to 0.5% by weight.

The vitamin C is present in an amount from about 0.5 to 30% by weight, preferably from about 3 to 10% and most preferably from about 5 to 10%. The cystine or other sulfur amino acid is present in an amount from about 0.25 to 5% by weight, preferably from about 0.25 to 1% and most preferably from about 0.25 to 0.5%. The mucopolysaccharide, when present, is present in an amount from about 0.05 to 10% by weight, preferably from about 0.05 to 2% and most preferably from about 0.05 to 0.5%.

The medication can be used to treat a wide variety of conditions. For example, in the reproductive tract it can be used to treat vaginitis and cervicitis. In the genitourinary tract, it can be used to treat urethral infections, especially the irritated bladder of schistosomiasis patients, and in the eyes it can be used to treat extropian eyelids, blepharitis, keratitis, and pinkeye and to prevent cataracts and diabetic retinopathy. On the skin, it can be used to treat burns, cuts, fever blisters, poison ivy, chigger bites, diaper rash, genital herpes blisters and even sunburn. The conditions listed above indicate the scope of the invention and are not meant to be limiting. Depending on the locus of the treatment and the method of application, the medication can be formulated in an appropriate water, oil or gel vehicle; spray, powder or medicated bandage, for example .

The following examples illustrate the invention.

EXAMPLE 1

Eighty Charles River variety white male rats, weighing about 150 g were divided randomly into 8 groups of 10 animals each, 2 control and 6 treatment groups:

C1--Control/burn only (After treatment these animals were kept out of the infectious area)

C2--Control/burn

T1--Burn/Ointment containing 2% ascorbic acid
T2--Burn/Ointment containing 2% zinc sulfate
T3--Burn/Ointment containing 2% cysteine
T4--Burn/Ointment containing 2% ascorbic acid and 2% zinc sulfate
T5--Burn/Ointment containing 2% ascorbic acid and 2% cysteine
T6--Burn/Ointment containing 2% ascorbic acid, 2% zinc sulfate and 2% cysteine.

Each animal was weighed, shaved and anesthetized with sodium pentabarbitol. They were placed on a steam outlet and exposed on the dorsal side for 20 seconds. The burned area measured 3".times.11/2" and the temperature of the skin was measured before and after the burn.

With the exception of the rats in group C1, the burn area of each rat was inoculated one hour after being burned with 2 ml of a 24 hour culture of Pseudomonas aeruginosa (diluted to 10.sup.7 /ml). A bacterial sample was obtained 24 hours post burn and treatment with ointment was begun. The animals were weighed weekly and cultures were obtained after 1 week, 2 weeks, 3 weeks and 4 weeks. The animals were sacrificed at the end of the 4th week and the skin, liver, kidney, adrenal and spleen were analyzed.

The results are shown in FIGS. 1 and 2 and in Table 1 and illustrate that the combination of zinc salt, vitamin C and cysteine was most effective in promoting wound healing as determined by measuring the percentage of new skin and granulation tissue in the burned area using a technique adapted from chromatography. More particularly, a piece of waxed paper was placed over the 3".times.11/2" burned area and the eschar and granulation tissue traced on the waxed paper as viewed therethrough. Percentages were determined by cutting out and weighing the various areas. Differences among the results in the treatment groups did not show up immediately since it takes time for the cells to form granuloma and for new cells to grow.

TABLE 1

Observation of 7-day, 14-day, and 21-day Photographs To Ascertain

the Percentages of New Skin and Granulation Tissue

Animal Number	New Skin Tissue	Granulation Tissue	New Granulation Tissue	New Granulation Tissue	New Granulation Tissue
	7TH DAY	14TH DAY	21ST DAY		
GROUP C.sub.1					
1	0%	0%	0%	1%	1%
3	0%	0%	0%	0%	5%
5	0%	0%	0%	0%	10%
7	0%	0%	0%	0%	0%
9	0%	0%	1%	5%	15%
-.chi.	0%	0%	0.2%	1.2%	6.2%
S.D. .+-.	0.00	0.00	0.00	0.45	2.17 6.30
S.E. .+-.	0.00	0.00	0.00	0.20	0.97 2.82
GROUP C.sub.2					
12	0%	0%	0%	7%	5% 35%
14	0%	1%	0%	3%	25% 25%
16	0%	0%	1%	5%	3% 32%
18	0%	0%	0%	0%	0% 10%
20	0%	0%	0%	8%	3% 22%
- .chi.	0%	0.2%	0.2%	4.6%	7.2%

24.8%

S.D. .+-.

0.00

0.45 0.45

3.21 10.11

9.78

S.E. .+-.

0.00

0.20 0.20

1.44 4.52

4.37

GROUP T.sub.1

21	0%	0%	3%	10%	50%	50%
23	0%	0%	3%	3%	5%	15%
25	0%	0%	0%	1%	30%	70%
27	0%	0%	0%	3%	20%	80%
29	0%	0%	5%	5%	20%	30%
-.chi.	0%	0%	2.2%			

4.4% 25% 49%

S.D. .+-.

0.00

0.00 2.17

3.44 16.58

27.02

S.E. .+-.

0.00

0.00 0.97

1.54 7.42

12.08

GROUP T.sub.2

32	0%	0%	0%	0%	10%	10%
34	0%	0%	0%	5%	5%	15%
36	0%	0%	5%	3%	25%	15%
38	0%	0%	1%	5%	30%	70%
40	0%	2%	5%	15%	45%	25%
-.chi.	0%	0.4%	2.2%			

5.6% 23% 27%

S.D. .+-.

0.00

0.83 2.59

5.64 16.05
24.65

S.E. .+-.
0.00
0.40 1.16
2.52 7.18
11.02

GROUP T.sub.3

41	--	--	10%	10%	40%	60%	
43	0%	0%	10%	5%	60%	40%	
45	0%	0%	11%	1%	30%	15%	
47	0%	10%	20%	40%	85%	15%	
49	0%	0%	0%	10%	30%	10%	
-.chi.	0%	2.5%	10.2%				
			13.2%	49%	28%		

S.D. .+-.
0.00
5.00 7.09
15.45 23.56
21.39

S.E. .+-.
0.00
2.50 3.17
6.91 10.54
9.57

GROUP T.sub.4

52	0%	0%	8%	2%	40%	15%	
54	0%	0%	10%	10%	40%	30%	
56	0%	0%	10%	0%	35%	15%	
58	0%	0%	13%	5%	15%	20%	
60	0%	0%	10%	0%	10%	5%	
-.chi.	0%	0%	10.2%				
			3.4%	28%	17%		

S.D. .+-.
0.00
0.00 1.79
4.22 14.40
9.08

S.E. .+-.
0.00

0.00 0.80
1.83 6.44
4.06

GROUP T.sub.5

62	0%	0%	10%	5%	60%	40%
63	0%	0%	11%	0%	50%	50%
65	0%	0%	8%	0%	60%	40%
67	0%	0%	6%	5%	40%	50%
69	0%	0%	10%	5%	50%	40%
-.chi.	0%	0%	9%	3%	52%	44%

S.D. .+-.
0.00
0.00 2.00
2.74 8.37
5.48

S.E. .+-.
0.00
0.00 0.89
1.22 3.74
2.45

GROUP T.sub.6

72	0%	0%	15%	0%	65%	35%
74	0%	0%	15%	0%	70%	30%
76	0%	0%	20%	0%	60%	40%
78	0%	0%	25%	0%	55%	45%
80	0%	0%	15%	10%	55%	45%
-.chi.	0%	0%	18%	2%	61%	39%

S.D. .+-.
0.00
0.00 4.47
4.47 6.52
6.52

S.E. .+-.
0.00
0.00 2.00
2.00 2.92
2.92

EXAMPLE 2

In order to document that mucopolysaccharide acts as a barrier, thereby preventing toxins on the skin surface from penetrating into the blood circulation system which otherwise leads to septicemia, thirty rats were divided into the following three groups:

C1--Control/Burn

T1--Burn/Ointment containing 2% zinc sulfate, 2% ascorbic acid and 2% cysteine

T2--Burn/Ointment containing 2% zinc sulfate, 2% ascorbic acid, 2% cysteine and 10% mucopolysaccharide extracted from aloe vera plant

Each animal was weighed, shaved and anesthetized with sodium pentabarbitol. They were placed on a steam outlet and exposed on the dorsal side to 60 seconds of steam (instead of 20 seconds as in the case of Example 1) which resulted in severe burn. The burned area measured 3".times.11/2" and the temperature of the skin was measured before and after the burn.

The burn area of each rat was inoculated, bacterial sample obtained, treatment begun, animals weighed, cultures obtained and animals sacrificed at the same intervals and in the same manner as described in Example 1.

All of the control animals died within one week due to septicemia which was documented by blood culture. In the treated group that received the combination of 2% zinc sulfate, 2% ascorbic acid, and 2% cysteine, six of the ten animals died between the second and third week after treatment. In the treated group where 10% mucopolysaccharide was added to the combination of 2% zinc sulfate, 2% ascorbic acid and 2% cysteine, none of the animals died.

EXAMPLE 3

Ten male rats, sexually mature, were divided into two groups. Group I was treated with Formula A and Group II was treated with Formula B. One hundred cc of each solution was formulated as follows:

Formula A Formula B

(Animals 1-5)

(Animals 6-10)

Zinc sulfate heptahydrate	2%	2%
Ascorbic acid	2%	2%
L-Cysteine	1%	--
Hydroxproline	--	1%
Water	100 cc	100 cc

At 9 a.m., an area three centimeters by six centimeters was shaved at the center of each animal's back. A wound incision was made, three centimeters in length, until the outer skin was separated from the body. The wound was irrigated with 0.25 ml of Formula A or B at 9 a.m. and again at 4 p.m. The animals were treated for another two days at 9 a.m. and 4 p.m. Observations were conducted at 8 hours after treatment, 24 hours after treatment, and on a daily basis for seven days. On the eighth day, photographs were taken, and after one month the animals were sacrificed, and histology samples were taken. Wounded skin samples and control samples were taken from each animal using Masson's Trichrome histochemical technique to document cell proliferation and new cell formation. Masson's Trichrome technique stains the nuclei black; cytoplasm, keratin, muscle fibers, and intercellular fibers red; and collagen blue. By observation documented by photographs and by pathological microscopic examination of the wounded skin as compared to non-wounded skin from the same animal used as a control for cytological comparison, it was determined that the animals in Group I had increased collagen formation, fibroblast cells and keratin over those in Group II. The number of nuclei were also counted as a measure of cell proliferation and new cell formation by comparing the animals in Group I with those in Group II. The number of nuclei in a one square-inch field reflected by a phase microscope on a TV screen per group was as follows:

Vitamin C + Zinc +			
Animal No.	Cysteine Nuclei/Square-Inch	Animal	Proline

	No.	Nuclei/Square-Inch	
1	88	6	80
2	93	7	79
3	86	8	83
4	97	9	77
5	94	10	81
-x	91.60	x	80.00
S.D. .+-.	4.51	S.D. .+-.	2.24
S.E. .+-.	2.01	S.E. .+-.	1.00

*Significantly different ($p < 0.01$)

In addition to having the indicated effect on epithelialization, the solution was noted to have the following effects:

1. Keeps the surface damp, thereby preventing damage by dehydration to the dermis and epidermis.
2. Allows free exchange of gases between wound surface and atmosphere, thereby increasing available oxygen.
3. Prevents accumulation of free fluid between the dressing and the wound, thereby decreasing the likelihood of infection.
4. Absorbs exudate and destroys bacteria.
5. Accelerates the formation of:

A. Fibroblast cells which secrete mucopolysaccharides, which contibute to fiber orientation and polymerization. The fibroblasts lay down and absorb collagen at the periphery of the open wound. It increases the micro-circulation of blood which carries oxygen needed to promote wound healing.

B. Collagen formation which is a unique amino acid containing 30% glycerine and 10% proline and hydroxyproline. Hydroxyproline and hydroxylysine appear to be unique components of animal collagen. Collagen aggregates immediately adjacent to the cell margins.

C. Keratin; the keratin layer of the skin serves as a barrier to noxious stimuli, which are constant problems of the integument.

6. Activates the occlusion of the open wound. The edges of the wound are evenly closed.

EXAMPLE 4

Seventeen men, ages 21-30, suffering from urethra pain, difficult urination and a burning sensation during urination were selected as subjects. The patients had a bacteria count from 10.sup.5 to 10.sup.6 /ml of urine and were single and sexually active, having more than one partner. They were treated with antibiotics, but the symptoms came back after 20-30 days and eight of the patients had frequent symptoms for 3 years.

An aqueous solution for urethra irrigation was made containing 3% ascorbic acid, 0.25% zinc present as zinc sulfate heptahydrate, 0.50% mucopolysaccharide and 0.25% pectin. A 3 ml disposable plastic syringe with a 20 guage needle one inch long having a bulb at the tip of the needle was used. The needle was lubricated with xylocaine local anesthesia and the urethra was irrigated by slowly administering 3 ml of the solution. Administration of the solution continued once a day for five days. After 2-3 days, the burning sensation disappeared. On the fifth day the bacteria count was less than 10.sup.3 which is normal.

For prevention, the patients were asked to irrigate once a month and were followed for 12 months. In this time, 15 of the patients did not have recurrent urethra infections. Two patients experienced blisters of a herpes infection on the glans penis and urethra opening 7 and 91/2 months after treatment. When the herpes blisters were irrigated twice a day for three days, the blisters started healing and pain and irritation stopped 24 hours after treatment.

EXAMPLE 5

One hundred and twenty-eight patients suffering from schistosomiasis of the bladder and a urinary tract infection were selected as subjects. They were from 22 to 48 years old, the concentration of bacteria was over 10.sup.6 /ml of urine and all of them had very painful urination.

The patients were first treated unsuccessfully with tetracycline antibiotics but the pain persisted and occasionally blood appeared in the urine.

An aqueous solution for bladder irrigation was made containing 3% by weight ascorbic acid, 0.25% zinc sulfate present as zinc sulfate heptahydrate, 0.50% mucopolysaccharide, 0.25% pectin and 0.15% cysteine. The bladder was irrigated three times a week for two

weeks. The blood in the urine disappeared after the second treatment and the pain of urination was eliminated after 3-4 treatments. After the sixth treatment, the bacteria in the urine was 10.sup.4, which is normal.

EXAMPLE 6

A vaginitis douche powder for menstruating women was made from a mixture of 2.5 g ascorbic acid, 1.25 g zinc sulfate heptahydrate, 0.5 g mucopolysaccharide (chrondroitin sulfate or hyaluronic acid) and 0.5 g polysaccharide (locust bean gum, xanthan gum or karaya gum). A douche solution was then made by dissolving the powder in 100 cc of sterilized water or a gel was made by dissolving the powder in 100 g of K-y gel.

A vaginitis douche powder for pre- and postmenopausal females was made by mixing 2 g ascorbic acid, 1 g sodium ascorbate, 0.5 g zinc sulfate heptahydrate, 0.5 g mucopolysaccharide (chrondroitin sulfate or hyaluronic acid) and 1.5 g polysaccharide (locust bean gum or pectin). The powder was dissolved in 100 cc water or 100 g K-y gel.

A flora douche was made for each of the above-mentioned types of subjects. For menstruating women, the flora douche included 3% by weight lactobacilli, 0.5% lactose, 1% glycogen and 0.5% pectin and for pre- and postmenopausal patients it included 3% by weight lactobacilli, 1% lactose, 3% glycogen, 0.5% pectin and 0.5% cysteine.

Eight female patients, 20-25 years of age who had been using tampons for at least two years, developed a burning sensation and itching during the last two days of menstruation. Five of the eight patients had ulceration on the orifice of the vagina, and in the remaining three patients, 2-3 ulcerations were also noted on the cervix in addition to the ulceration on the vagina. Examination of these patients revealed there were no pathogens present, i.e., gonorrhea, Haemophilus bacteria, yeast or Trichomonas, leading to the suspicion that the irritation was being caused by physical objects such as tampons or adhered toilet paper.

These patients were followed for six months and given the vaginitis douche described above to use twice daily starting from the third day of menstrual bleeding; then they were inoculated with a warm solution of flora douche. They followed this treatment for six months. While they continued to use tampons, no ulceration occurred and there was no discomforting irritation or itching.

EXAMPLE 7

Itching is a disagreeable sensation produced by the action of stimuli of a harmful nature on the skin surface. It is a signal of actual or potential danger to the skin defined as an expression in consciousness of the response of scratching or rubbing. The itch perception is usually accompanied by a feeling or emotional state so that the entire experience is apt to be complex in nature. Unfortunately, pruritis is the most outstanding and characteristic sensory feature of many skin diseases and the motor response it evokes, if not controlled, leads only to further damage of the skin surface often with perpetuation and intensification of the unpleasant and even intolerable symptom.

Thirty-one patients complaining of itching due to mosquito bite, poison ivy, chigger bite or irritated genitalia were treated with Itching Cream composed of 2.5% sodium ascorbate, 2.5% ascorbic acid, 2.0% zinc sulfate heptahydrate, 3.0% cysteine and 90.0% HEB Cream as a base. The duration of treatment was as follows: Mosquito bite, one treatment or a second treatment after 3 hours; poison ivy, two treatments daily for 4 days; chigger bite, two treatments daily for 2 days and irritated genitalia, three treatments daily for 4-5 days.

The results are shown in Table 2 below.

TABLE 2

Patient No.	Time Lapse (Minutes) Between Drug Application and Stoppage of Itch
Mosquito Bite	
1	10
2	16
3	18
4	26
5	9
6	23
-x	17.00
S.D.	.+-. 6.81
S.E.	.+-. 2.78
Poison Ivy	

7	12
8	18
9	18
10	19
11	24
12	17
13	27
14	34
-x	21.13
S.D.	.+-. 6.30
S.E.	.+-. 2.44

Chigger Bite

15	15
16	11
17	9
18	16
-x	12.75
S.D.	.+-. 3.30
S.E.	.+-. 1.65

Irritated

19	23

Genitalia

20	16
21	11
22	8
23	7
24	13
25	18
26	8
27	5
28	12
29	16
30	14
31	19
-x	13.08
S.D.	.+-. 5.28
S.E.	.+-. 1.47

EXAMPLE 8

Sixteen men, 21-36 years of age, complaining of balanitis were subjects. The different forms of balanitis noticed in these patients included:

A. Inflammation of the penile skin (balanoposthitis), inflammation occurring in the glans and the mucous surface of the prepuce;

B. Infective forms, erythma of the glans, the coronoal sulcus, and inner surface of the prepuce; candidial balanitis occurring after intercourse with an infected partner;

C. Amoebic balanitis occurring in patients who practiced anal intercourse; severe inflammation of the prepuce and severe irritation of the glans and/or skin ulceration on the glans.

The cause of balanitis in each of the patients is summarized in Table 3.

TABLE 3

Patient No.	Cause
1-5	Yeast infection of the female partner
6-8	Cervicitis of female partner
9	Zipper injury
10, 11	Clothing friction
12-14	Long foreskin combined with poor hygiene
15, 16	Anal intercourse

Patients were advised to wash the penis twice daily for 10 minutes using a male hygienic solution composed of the following: 10.0% mucopolysaccharide from aloe vera, 3.0% ascorbic acid, 2.0% sodium ascorbate, 0.5% zinc sulfate heptahydrate, 1.0 cysteine in 1000 cc of distilled water.

The penis was placed in a cup, 6 inches in length and 21/2 inches in diameter, connected to a battery-operated pump to circulate the solution. The movement of the solution by the pump gently scrubbed the penis, removing dead cells, debris and hair that had adhered to the skin.

Patients were treated 3 to 5 days depending on their condition. After 5 days, the irritation had stopped and the ulceration began healing. Patients were advised to use the male hygienic solution and cup after having sexual relations. The suggested regimen was to use the solution and cup for 10 minutes, then rinse the penis and dry it thoroughly with a disposable towel.

Patients were followed for a 3-month period during which time no recurrent inflammation occurred.

EXAMPLE 9

Five women, 19-35 years of age, complaining of odor from vaginal secretion and all of which had tried over-the-counter and prescribed douches (Betadine solution) were subjects. In order to obtain vaginal secretion, commercial tampons were reduced to 1 cm in length, washed in hot methanol in a Soxhlet extractor for 2 hours, dried at 110 degrees C. and hermetically sealed in polyethylene bags. This procedure removed waxes and other extractable matter which would have interfaced later on with the analysis and also protected the tampons from contamination. Each subject was provided with a convenient kit containing 5 tampons (one extra) and 4 numbered and snap-cap bottles each containing 20 ml of methanol. Subjects were instructed to wear each tampon in the usual way and, on removal, to dip it immediately into the bottle provided.

Patients were instructed to insert the tampons on four consecutive evenings, to wear each tampon 12 hours, and then to remove each tampon the following morning in order to complete a 24-hour time period between insertion of tampons. After removal of the second, third and fourth tampons at their respectively scheduled times, the vagina was irrigated with one liter of the following solution: 3.0% sodium ascorbate, 2.0% ascorbic acid, 1.5% zinc sulfate heptahydrate, 1.0% cysteine and 1000 cc distilled water.

The use of very small tampons for only 12 hours out of each 24 hours helped to minimize any disturbance to the normal bacterial flora and immersion of tampons in methanol immediately after removal stopped bacterial action.

Upon arrival back in the laboratory, tampons were packed individually into glass columns and washed with methanol in chromatographic fashion. Eluates were combined with methanol from the sample bottle, mixed with 100 .mu.l N/10 sodium hydroxide to reduce the volatility of the fatty acids, and evaporated

to dryness. Residues were taken up in 1 ml water, washed with 4 ml ether (to remove basic and neutral components), and the aqueous layers were acidified (below pH 2.0) and extracted with 4 ml ether containing n-pentanol as a concentration marker. Extracts were concentrated to 50 .mu.l and analyzed on 10% FFAP columns in a Perkin Elmer gas chromatograph; programmed from 50 to 220 degrees C. at 5 degrees/min. Peaks were identified by absolute retention time, retention time relative to the concentration marker (n-pentanol), and retention time relative to the other members of the acid series. In addition, peaks produced by free fatty acids on FFAP columns have a characteristic tail not shown by less polar compounds. Other substances which might have interfered with chromatographic identifications would have been been removed during the sample preparation. Peak areas were determined manually and expressed as ratios of the area of the n-pentanol peak. After tampon collection 3 times and 3 irrigation treatments, the foul smell decreased and volatile fatty acids also decreased significantly ($p<0.001$) as expressed in Table 4.

TABLE 4

VOLATILE FATTY ACIDS (C.sub.2 --C.sub.5) CONTENT (.mu.g) OF VAGINAL SECRETION BEFORE AND AFTER IRRIGATION TREATMENT

Patient No.	Before Irrigation*	After 1st Irrigation	After 2nd Irrigation	After 3rd Irrigation
A	162.4	110.8	60.6	30.8
B	140.1	130.6	90.9	46.7
C	110.5	105.3	80.7	42.3
D	179.8	110.9	70.2	36.4
E	145.2	120.3	81.4	48.6
-x	147.6	115.58 (1)	76.76 (2)	40.96 (3)
S.D. .+-.				

	25.35	9.98	11.63	7.37
S.E. .+-.				
	11.61	4.46	5.20	3.29

*Control

(1), (2), and (3) Significantly different from Control ($p < 0.001$)

EXAMPLE 10

A pinkeye powder was made from 5 g vitamin C, 1 g zinc sulfate heptahydrate, 100 mg keratin sulfate and 2 g cysteine and packaged in aluminum foil for solution in 100 ml of sterilized water to which is added 2% by weight pectin which makes the solution stick to the eyes and 0.05% benzalkonium chloride which acts as a preservative. Two hundred and eighty infected cattle, showing clinical signs of conjunctival hyperemia and edema, particularly of the bulbar conjunctiva and eighty of which showed an opaque area elevated from the cornea, were treated with the above-mentioned eye spray, by spraying 5 strokes into each eye for 2 days. On the second day, some improvement was noticed and the lesions showed an indication of healing and redness decreased significantly. After 5-7 days, the eyes of 269 of the animals were normal. The remaining 11 animals had ulceration in the cornea and eyelid and corneal opacity (abscess varying from a pale yellow to white) and there was a marked circumcorneal congestion of the conjunctival vessels. These 11 animals were treated for 4 days and became normal after 10 days.

One hundred and fifty other infected cattle were treated with thymol blue but only 30 of them showed improvement after 5-7 days. Another group of 148 infected animals were treated with 1% penicillin and only 7% showed an improvement after 5-7 days.

EXAMPLE 10

In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained. As various changes could be made in the above compositions and methods without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense.

* * * * *